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EXTRACTION OF CHLORIDE FROM RAT-URINE SAMPLES FOR CHLORINE-36 ANALYSIS

LOS ALAMOS

QUALITY PROGRAM

APPROVAL FOR RELEASE

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Los Alamos
Yucca Mountain Site
Characterization Project

HISTORY OF REVISION

REVISION NO.	EFFECTIVE DATE	PAGES REVISED	REASON FOR CHANGE
R0	07/31/95	N/A	Initial procedure.
R1	10/16/96	All	Major revision of procedural steps to improve sample quality.

EXTRACTION OF CHLORIDE FROM RAT URINE SAMPLES FOR CHLORINE-36 ANALYSIS

1.0 PURPOSE

This detailed technical procedure (DP) describes the process for extracting chloride from packrat urine samples in order to prepare them for chlorine-36 analysis for the Yucca Mountain Site Characterization Project (YMP) Water Movement Test.

2.0 SCOPE

This DP applies to Los Alamos and Los Alamos subcontractor personnel (hereinafter referred to as users) who process packrat urine samples for chlorine-36 analysis as part of the YMP Water Movement Test study .

3.0 REFERENCES

LANL-YMP-QP-02.7, Personnel Training
LANL-YMP-QP-03.5, Documenting Scientific Investigations
LANL-YMP-QP-17.6, Records Management
LANL-CST-DP-94, Using Ion Chromatography to Determine Chloride and Bromide Concentrations
LANL-CST-DP-97, Preparation of Carrier Solution for Chlorine-36 Samples
LANL-CST-DP-103, Identification, Storage, and Handling of Samples for the Water Movement Test

4.0 DEFINITIONS

4.1 Accelerator Mass Spectrometry

Accelerator mass spectrometry (AMS) is the analytical method used to determine the atomic ratio of chlorine-36 to total chlorine in a sample of silver chloride.

4.2 Amberat

Amberat is crystallized packrat urine.

4.3 Midden

A packrat midden is a packrat nest that includes a section containing droppings and urine. Usually a midden has been occupied by several generations of packrats, in which case the droppings will form layers in which younger layers overlie older ones.

4.4 Reagent Water

Reagent water is water that has been analytically purified to remove impurities so that the resistivity is greater than 17.5 megohm cm.

4.5 Process Blank

The purpose of a process blank is to test for the presence of chlorine-36 contamination in processing samples for chlorine-36 analysis. A process blank is required to accompany each sample suite. It consists of taking a volume of reagent water containing carrier chloride (prepared per DP-97) through the same processes, and using the same materials, as are used to prepare the actual samples. The presence of chlorine-36 in the process blank may indicate a problem with chlorine-36 contamination.

5.0 RESPONSIBILITIES

The following YMP employees are responsible for implementing the activities in this DP:

- Principal Investigator (PI)
- User

6.0 PROCEDURE

The use of this procedure must be controlled as follows:

- If this procedure cannot be implemented as written, YMP personnel should notify appropriate supervision. If it is determined that a portion of the work cannot be accomplished as described in this DP, or would result in an undesirable situation, that portion of the work will be stopped and not resumed until this procedure is modified replaced by a new document, or current work proactive is documented in accordance with QP-03.5, Section 6.1.6.
- Employees may use copies of this procedure printed from the controlled document electronic file; however, employees are responsible for assuring that the correct revision of this procedure is used.
- When this procedure becomes obsolete or superseded, it must be destroyed or marked “superseded” to ensure that this document is not used to perform work.

6.1 Principle

The initial ratio of chlorine-36 to total chlorine in infiltrating water is a parameter used to estimate groundwater travel times in the unsaturated zone at Yucca

Mountain and may have varied significantly over the past few hundred thousand years due to changes in atmospheric production rates and in chloride deposition rates. It is hypothesized that changes for the past 50,000 years may be reconstructed by measuring chlorine isotopic ratios of packrat urine from abandoned packrat middens that have been dated by the carbon-14 method.

6.2 Equipment and Hardware/Software

Equipment needed to conduct this DP is listed below. Not all of the items are absolutely necessary.

- Water purification system capable of producing reagent water with a resistivity greater than or equal to 17.5 megohm cm (calibration not required; see subsection 6.3.2)
- Centrifuge (calibration not required)
- Convection oven capable of maintaining a temperature in the range of 60 to 90 C (calibration of oven thermometer not required)
- Muffle furnace capable of maintaining a temperature of approximately 500 to 600 C (calibration of oven thermometer not required)
- Hot water bath (calibration not required)
- Balance with a capacity of 0.1 to 200 g (calibration not required; see subsection 6.3.2)

6.2.1 Equipment Malfunctions

Any equipment malfunction occurring during implementation of this procedure will be detectable in the course of conducting work; therefore, it is not expected to have a detrimental effect on the final results. The water purification system has a meter that indicates the resistivity of the purified water. This meter is checked prior to use by the user to verify that the resistivity is greater than or equal to 17.5 megohm cm. In addition, the purpose of the process blank (subsection 4.5) is to alert the user to contamination introduced by sources such as a malfunctioning water purification system. If a problem arises which can be considered a potential source of error or uncertainty for the results, then it is addressed in subsection 6.7.

6.2.2 Safety Considerations

Good laboratory and scientific practices are used to protect against injury. Applicable LANL and/or LANL-subcontractor safety practices for conducting laboratory work are followed.

6.2.3 Special Handling

See subsection 6.3.3.

6.2.4 Laboratory Materials and Chemicals

6.2.4.1 Materials and Supplies

Laboratory glassware and plasticware are used for preparation and storage of reagent solutions and samples.

6.2.4.2 Reagent Chemicals

- Reagent water
- Nitric acid (HNO_3), ACS reagent-grade
- Silver nitrate ($AgNO_3$), ACS reagent-grade
- Barium nitrate ($Ba(NO_3)_2$), ACS reagent-grade
- Ammonium hydroxide (NH_4OH), ACS reagent-grade
- Carrier solution, prepared according to DP-97

6.3 Preparatory Verification

6.3.1 Hold Points (N / A)

6.3.2 Calibration

None of the equipment listed in subsection 6.2 requires calibration. The meter on the water purification system is an integral part of the water purification system. Meter failure is noted by a “zero” reading, at which time the meter is replaced by the vendor. The balance does not require calibration because it is used only to obtain approximate sample weights in order to ensure adequate sample size for analysis.

6.3.3 Environmental Conditions

The most critical part of the process described in this DP is to scrupulously avoid introducing other sources of chloride during processing. Hence, careful cleaning before and during the process, to avoid contamination with any sources of chloride other than the midden itself, is essential. The purpose of the process blank (subsection 4.5) is to monitor for the presence of contamination.

6.4 Control of Samples

It is imperative that sample identification and control be sufficient to trace a sample and its derivative from its original field location to the point of analysis and that the integrity of the sample be safeguarded during the entire analytical process. Consequently, users must be trained to DP-103 before they can work with samples analytically and they must also follow guidelines set forth in that document for sample management.

6.5 Implementing Procedure

The user implements the steps described throughout subsections 6.5.

6.5.1 Break up a portion of the sample and select a subsample that consists of as pure amberat as possible. The samples are typically very well-indurated and can often be broken only with a rock hammer or another such instrument. If the sample is of suitable size, a hacksaw may provide a better means of selecting a subsample. If the sample is large, efforts should be taken to select a precise subsample, i.e., from a well-defined layer, so as to limit complications due to possible spatial variations within the midden.

6.5.2 Set aside sufficient sample for radiocarbon (^{14}C) dating (about 5 g midden material) as well as for the following procedure (about 10 g midden material, sufficient to contain about 40 mg chloride). Samples for ^{14}C dating are best selected using a microscope to pick out pieces of relatively pure amberat. These samples should consist of a split of the same portion to ensure that ^{14}C and ^{36}Cl analyses are obtained from the same material.

6.5.3 Place the samples in clean covered crucibles marked with the sample number. Create a blank containing ~ 20 mg Cl by evaporating an appropriate volume of carrier solution (prepared according to DP-97) in a crucible.

6.5.4 Ash the samples and blank by heating the crucibles in a suitable oven. Care should be taken that the oven is relatively clean before each use. Particulate matter clinging to the walls of the oven, which may contain chloride, may be removed by vacuuming and/or wiping with a wet cloth. The layout of the samples in the oven must also be recorded before heating; any markings on the crucibles will burn off during heating. Heat the samples at 500 - 600° for at least two hours.

6.5.5 After heating, open the door of the oven and allow to cool for ~1 hour before removing for further cooling. This should cool the crucibles

enough to permit marking as they are removed from the oven, using the prerecorded sample layout. With a clean spatula, scrape the ashes from each crucible into a container large enough to contain the sample and approximately 40 mL of solution to be added in steps 6.5.6 and 6.5.8. The process blank must be removed by several rinses with reagent water. Mark each container and cap with the sample number.

6.5.6 Add no more than ~30 mL of reagent water to each sample. It is desirable that the solution volume will fit easily into a 50 mL centrifuge tube in step 6.5.9, after the addition of $Ba(NO_3)_2$ in step 6.5.8, and leaving room for the addition of HNO_3 and $AgNO_3$ in steps 6.5.11 and 6.5.12.

6.5.7 Set on rotating shaker table at a low speed and leave overnight to ensure that all the available chloride goes into solution.

6.5.8 Add ~10 mL of warm saturated $Ba(NO_3)_2$ solution to the leaching containers. A white amorphous precipitate should form easily. Enough solution should be added that no more precipitate forms when additional $Ba(NO_3)_2$ is added.

Note: The purpose of this step is to remove sulfur by precipitating it in the form of $BaSO_4$. Warming the $Ba(NO_3)_2$ solution increases its solubility and hence helps to minimize the volume of liquid that needs to be added to the sample. Although adding the $Ba(NO_3)_2$ at this stage instead of a later one increases the amount needed, it also often reduces the quantity of unwanted precipitates that initially form.

6.5.9 Filter the sample using a clean Millipore filter setup, 0.45 -micron filter(s), and labeled filtration flasks. Discard the precipitates and transfer the liquid samples to 50 -mL centrifuge tubes. If sufficient chloride is available, some of the solution may be saved in a separate container as a precaution against processing errors.

6.5.10 Measure the chloride concentration of the sample if desired, such as by ion chromatography (following DP-94) or ion-specific electrode (documented per QP-03.5).

6.5.11 Add sufficient concentrated HNO_3 to the samples to bring the pH to ~ 2 and check for formation of a precipitate. If a precipitate develops, separate and discard it by either filtering or centrifuging the samples, pouring off the supernatant into a clean container or into the filtration flask from subsection 6.5.9.

6.5.12 Add $AgNO_3$ solution (at an approximate strength of 1M to 2M) in the proportion of 1.2 to 1.5 mole Ag per mole Cl in the sample. The precise amount of $AgNO_3$ added is not critical and is chosen to ensure that sufficient Ag is available to combine with the sample Cl in the sample without adding too much excess Ag. Alternatively, if the Cl

concentration is not known, then slowly add dilute $AgNO_3$ until a AgCl precipitate no longer forms upon addition. Place the sample in a warm dark area for 2 to 3 hours to allow the AgCl precipitate to coagulate and settle.

Note: The molarity of the $AgNO_3$ solution is not critical, but the level suggested above is advantageous in that it minimizes the volume of solution that must be added to the sample, which is usually highly concentrated with respect to chloride.

6.5.13 Centrifuge the solution for several minutes, then pour off the supernatant, retaining the solid AgCl.

6.5.14 Dissolve the AgCl precipitate in ~5 mL of concentrated NH_4OH. Add ~1 mL of saturated $Ba(NO_3)_2$ solution to precipitate $BaSO_4$ and allow to sit for at least one hour (at least 8 hours if this is the last time through this step).

6.5.15 Discard any precipitate remaining in the samples that does not dissolve with addition of more NH_4OH. Do this by first centrifuging the samples at high speed, then decanting or pipetting the solutions into a separate set of clean, labeled, 50-mL centrifuge tubes. Discard the precipitate from the first tubes and clean them with dilute HNO_3 and reagent water.

6.5.16 Slowly add dilute HNO_3 to the sample solutions until all of the dissolved AgCl has reprecipitated. Centrifuge and decant, retaining the solid AgCl.

Note: The amount of HNO_3 required is slightly greater than the amount required to neutralize the solution. The addition of dilute HNO_3 avoids the vigorous exothermic reaction that results when concentrated acid is mixed with concentrated base.

6.5.17 Repeat subsections 6.5.14 through 6.5.16 at least two times to remove as many contaminants as possible. Each repetition of this procedure should cause the color of the AgCl precipitate to become increasingly lighter as the AgCl purity increases.

6.5.18 Again add sufficient dilute HNO_3 to precipitate AgCl and let stand for approximately 2 hours. Decant off the acidic solution and rinse the AgCl three times in reagent water. Contact the AMS laboratory which will be analyzing the sample for $^{36}C1$as to the appropriate method of packing the samples before proceeding with the following subsections. In some cases, the AMS laboratory may accept damp samples, and packing may consist solely of submitting the sample in an appropriately clean container.

6.5.19 The remaining subsections 6.5.19 through 6.5.22 describe one method of drying the samples for submission to the AMS laboratory for analysis. Prepare a set of watch glasses for drying the precipitates by thoroughly cleaning the glasses with soap and brush and then soaking them at least one hour in concentrated NH_4OH. Rinse the glasses thoroughly in reagent water, and dry and label them with an indelible marker. If desired, record the mass of each of the watch glasses so that sample masses may be easily determined.

6.5.20 Transfer the AgCl precipitate onto a clean, pre-labeled watch glass. Remove excess water using a small glass pipette. Cover the glass with aluminum foil, leaving several holes near the edges to allow water vapor to escape. Place the sample in the oven for 24 hours. Set the temperature to approximately 60°C.

6.5.21 Remove the samples from the oven; let cool fo r several minutes and then weigh each sample (on the watch glass) if it is desired to determine the mass of AgCl produced.

6.5.22 Using a clean metal spatula, scrape the AgCl into a loose pile on the watch glass and carefully transfer to a clean, pre-labeled vial.

6.6 Data Acquisition and Reduction

No masses or volumes are required to be recorded for this DP. Where such measurements are mentioned in the processing steps listed above, the intent is only to provide rough guidelines for the quantities of reagents to be added, or for estimates of the yield of a particular sample. The accuracy of those measurements do not affect the quality of the $^{36}Cl/Cl$ analysis.

6.7 Potential Sources of Error and Uncertainty

Attention to labels on sample containers and in recording data reduces error in mislabeling. The possibility of cross-contamination is reduced by working in a clean environment. If a problem arises which can be considered a potential source of error or uncertainty for the results, then it is documented in the laboratory notebook or logbook in accordance with QP-03.5.

7.0 RECORDS

Proper execution of this DP results in entries into a scientific notebook or logbook. Attachment 1 lists the information required to be documented. Entries are made in accordance with QP-03.5. The notebooks or logbooks are submitted as record packages to a Records Processing Center in accordance with QP-17.6.

8.0 ACCEPTANCE CRITERIA

The criteria showing that this procedure has been properly implemented are the entries generated in Section 7.0.

9.0 TRAINING

This DP requires "read-only" training. Training of personnel to this DP is documented pursuant to QP-02.7.

10.0 ATTACHMENTS

Attachment 1: Checklist of Laboratory Notebook Entries (1 page)

CHECKLIST OF LABORATORY NOTEBOOK ENTIRES

Initial descriptive information is entered in the laboratory notebook as appropriate prior to starting a technical procedure and on a continuing basis as experimental and procedural changes dictate. These entries include:

- reference to this DP, including revision number

Required entries are as follows:

- date of sample preparation
- unique sample identification number assigned per DP-103
- chemical reagent, manufacturer or supplier and lot number
- user's signature and date

Finally, the user must record problems, if any, which could be considered potential sources of error or uncertainty for the results.